page 11, line 3; and iv) page 14, lines 4-9 and page 15, lines 11-15.

Support for new claim 30 and 31, which specify a limitation of the number of amino acids encoded by the inserted random nucleotides, can be found in the specification on page 10, line 36, and on page 8, line 32.

New independent claims 34 and 35 are equivalent to claim 1 but include the limitations of dependent claims 25-26, respectively.

New independent claims 32, 33, 36, and 42 are equivalent to claim 1 but incorporate further alternative limitations.

Specifically, claim 32 includes the limitation that the detection of phenotypically altered cells is not done by means of capture of the expression product with a ligand. Support is found in the specification on page 2, lines 19, through page 3, line 21.

Claim 33 includes the limitation that the alteration in the phenotypic trait is ascribable to the expression products affecting biologically functions of the cell which have influence on the

preselected phenotypic trait. Support is found in the specification in the paragraph bridging pages 3 and 4.

Claim 36 simply includes the limitation that the expression products are confined to the intracellular phase. Support is found in the specification on page 3, lines 35-36, page 6, lines 6-9, page 7, last paragraph, page 14, last paragraph, and Example 3.

Claim 42 includes the limitation that the phenotypic trait screened for is one which is observable in the identical eukaryotic cells, even before transduction. Support can be found in Example 2 and Examples 3-4.

Dependent claim 37 is supported by original claim 2; dependent claim 38 is supported by original claim 19; dependent claim 39 is supported by original claim 9; independent claim 40 is supported by original claim 27; and dependent claim 41 limits the scope of claim 1 by making step (f) mandatory and adds that the ligand molecule is a molecule of the identical cells.

Before addressing the Examiner's grounds for rejection, Applicants submit the following summary of the presently claimed invention. The present invention relates to a novel method which

allows identification of putative drug leads and drug targets. This goal is achieved by expressing random DNA sequences in transduced eukaryotic cells. Some of the expression products will eventually interfere with biochemical/biological processes in the transduced cells and these changes can be detected by screening the transduced cells for alterations in the phenotype. Hence, the method allows for detection of indirect effects exerted by the random expression products so there is no need for a known ligand in the screening assay.

By using random DNA fragments it is obtained that the expression products are not restricted to naturally occurring expression products and therefor the number of interactions which can be detected by far exceeds the number which can be found by screening of cDNA libraries. Also, a high degree of flexibility is provided, since the length of the expressed sequences can be controlled, thus allowing production of small peptides and thereby facilitating the identification of the biologically relevant part of the molecule (something which would normally be difficult in screening of cDNA libraries, where the expressed cDNA is typically

detected because of the "native" action of the protein encoded by the cDNA). Finally, the interactions which are identified by the method of the invention are in general simple binding interactions between target molecules in the transduced cells and the random expression products, whereas screening of cDNA libraries will identify fully functional full-length proteins.

The gist and crux of the present invention resides in the present inventors' realization that short peptides and ribonucleic acids are capable of interfering with biochemical processes which take place inside cells. Consequently, the inventors therefore realized that if it is possible to obtain expression inside cells, in a controlled manner, of random DNA sequences, the expression products thereof would be able to interfere with the biochemistry of the cells, e.g., as a consequence of simple binding between the expression product and an unknown target inside the cell.

NON-ART BASED REJECTIONS

The Examiner has rejected claim 3 under 35 U.S.C. § 112, first paragraph, for containing subject matter which is not described in

the specification in such a way as to enable a person of skill in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claim 3 as amended now recites --random codon synthesis-- instead of "...codon split synthesis...". The support for the use of this term can be found in Example 1 on page 10, lines 21-22. In fact, the technology as such is described in a manner which is completely enabling for the skilled artisan in the text of Example 1, line 18 on page 10, through line 3 on page 11. Withdrawal of the rejection is therefore deemed appropriate.

The Examiner has rejected claims 1-4, 6-7, 9, 14-22 and 25-29 under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention.

Applicants address each of the Examiner's specific rejections in the order raised, as follows:

The Examiner has rejected claim 1 for the use of the expression "...changed a certain phenotypic trait...". As partly suggested by the Examiner, the claim has been rephrased to recite

--altered a preselected phenotypic trait--. The term "preselected" has been introduced in order to indicate that the screening aims at detecting a predefined type of change in the "positive" cell.

The Examiner's rejection of claim 1 in view of the language "synthetic random DNA sequences, in which restrictions upon the randomness may be introduced..." has been dealt with by splitting original possibility (ii) into 4 separate possibilities (ii)-(v). Hence, it should now be clear in what manner the randomness of the random DNA fragments is restricted, noting however that the DNA sequences are still random.

The term "...characterized in that..." has been deleted from the claims and the term "...wherein..." has been inserted instead.

The term "...like..." has been exchanged with "defined in..." to overcome the Examiner's rejection.

The term "...biologically active..." has been deleted from the indicated positions in the claims. The claims now recite that the ribonucleic acids used in steps (g) and (h) are those which effect an alteration in the preselected phenotypic trait.

In claim 2, the language has been amended to recite that the peptide sequence is inserted into or fused to a protein amino acid sequence, thus avoiding the word "introduced."

The wording of both of claims 3-4 has been amended so as to delete the use of the expression "...the random peptide library...".

Claim 5 has been amended by deleting "...the principle of..." as suggested by the Examiner. At the same time, the reference to "...the library..." has been deleted and replaced with the expression ---the totally or partly random DNA sequences-- which has antecedent basis in claim 1.

Claims 6-7 have been rephrased so as to render clear what method steps are involved in the two claims. Hence, the word "use" has been deleted in both claims.

Claim 9 has been amended to recite "... a vector selected from a retroviral vector or a vaccinia virus vector".

In claim 14, the definite article has been deleted in "...the virus packaging cells...".

In claim 15, the expression "...new target cells..." has been deleted and replaced with --further cells--.

In claim 16, the expression "...the PBS..." has been replaced with --...a primer binding site (PBS)--.

Claim 17 has been rephrased so as to render clear that transfection is performed on a cell which carries material derived from a vector which encodes one single transcript which in turn is translated into the three proteins gag-pol, a selectable marker, and env. The expression --selectable marker-- replaces the term "...drug resistance gene...". The latter is not a translation product, and moreover, drug resistance is one of several selectable markers which can be used in a packaging cells, for instance see page 12, lines 15-23 of the specification.

Claim 18 has been amended so as to render clear that a semi-packaging cell line is transfected with a corresponding minivirus/vector thereby enabling expression of the vector's genetic material after transduction but not after transfection. See the description on page 13, lines 23-37.

Claim 20 has been amended so as to render clear that a purification tag is attached to the expressed peptide so as to enable isolation of either the expression product itself or the cell molecule with which the expression product interacts.

Claim 22 has also been amended with a view to clarification: It should now appear clear that the random DNA sequences are either inserted into or fused to a DNA sequence which encodes a protein which is expressed simultaneously from the vectors.

In claim 19, the non-limiting phrase "...such as e.g. glycosylation sites and anchor residues.." has been deleted. Introduction of glycosylation sites and anchor residues is now the subject matter of claim 38.

In claim 20, the term "...biologically active protein..." has been replaced with --peptide effecting the phenotypic alteration--.

Further, the expression "...the target protein causing the biological activity..." has been replaced with --molecule with which the peptide interacts--.

Claim 21 has been amended by deleting the expression "...introduced DNA in such a way that they are fused to the..." and replacing it with --vectors in the form of fusion partners to--.

Further, the amendment to claim 21 encompass the deletion of the definite article in "...the random peptides...".

In claim 21, the definite article in "...the expressed proteins..." has also been deleted.

Claim 24 has been amended to recite that the protein is a heavy and/or light chain of an antibody molecule or a part thereof. Hence, the use of the language "...derived from..." has been deleted.

The deletion of claims 27-29 render the rejections of these claims moot. As for claims 25-26, these have been rephrased so as to specify the phenotypic trait screened for (complexes between epitopes and MHC molecules in claim 25 and surface expression of a protein in claim 26).

In view of the above comments and amendments, applicants request reconsideration and withdrawal of the rejection.

ART BASED REJECTIONS

The Examiner has cited Kay et al. under 35 U.S.C. § 102(e) as anticipating claims 1-2, 4-5, 8-11, 15-16, 20, 22-24 and 27.

In order for a reference to take away novelty of a claimed invention is must disclose each and every limitation of the claimed invention. However, Kay et al. does not disclose each and every limitation of present claim 1 and therefore cannot anticipate the presently claimed invention.

It is of course true that successful introduction of heterologous genetic material which is expressed and detected in an ELISA does introduce a phenotypic change, and therefore it cannot be denied that Kay et al. Screens for a phenotypic alteration. But this is not the only essential feature of present claim 1.

As was the case for original claim 1, present claim 1 calls for transduction of vectors into eukaryotic cells. In the art, the term "transduction" is defined as "transfer of genetic information from one cell to another by way of a viral vector," the enclosed copy of the search results for a search after the term "transduction" in the Biotech Life Science Dictionary on the

Internet, http://biotech.chem.indiana,edu/pages/dictionary.html. As further evidence, we also enclose a copy of page 109 in the textbook "Biology of Microorganisms," 5th edition, 1988, by Thomas D. Brock and Michael T. Madigan, where a similar definition is given in the left column, lines 28-29.

Nowhere in Kay et al. is transduction of eukaryotic cells described. In fact, the only place where we have been able to identify any mentioning of eukaryotic expression systems is in column 28, last line. The only techniques for transfer of genetic material which are described in Kay et al. are transfection techniques, and of these electroporation is specifically exemplified.

Further, apart from the fact that Kay et al. does not disclose the use of transduction methods, we have not been able to locate a disclosure in Kay et al. where it is taught that the method described therein can result in transfection of one single vector per eukaryotic cell or a limited number of vectors per cell; the commonly used transfection methods such as electroporation does not allow for such control of the "rate of success" in eukaryotic

system; in fact, this is a well-known problem when working with eukaryotes, where numbers in the order of 1000 transfections are seen per cell.

It should be noted that transfection methods are sometimes used as part of the strategy in the present invention, but the step of transducing cells is mandatory. When transfection is used according to the present invention, this is done in order to prepare packaging cells which subsequently produce infectious virus particles. These virus particles are in turn used in the mandatory transduction step, where substantially identical cells are infected.

In conclusion, since claim 1 is not anticipated by Kay et al., the remaining rejections under 35 U.S.C. § 102 of the claims dependent on claim 1 are moot.

At any rate, the teachings of Kay et al. are very far from the gist and crux of the present invention. The methods disclosed in Kay et al. exclusively enable identification of TSARs which bind to "ligands of choice" (see column 30, first 3 lines in section 5.2), and as such Kay et al. suffers the same drawbacks as do the

techniques described in the present specification, page 2, line 19, through page 3, lines 8: It is necessary to use a known ligand in order to identify a TSAR. The contribution to the art provided by Kay et al. is in fact an improvement to the techniques described on pages 2-3 in the present specification, i.e., the provision of a means for producing random libraries of very long nucleic acid fragments.

In contract, the present invention provides for a method which allows for the identification of biologically active peptides or ribonucleic acids encoded by a library of random DNA sequences, wherein the identification does not rely on the presence of a ligand of choice. Rather, the present invention also enables the identification of ligands. This is done by preselecting a phenotypic trait, the alterations of which are of interest, and then screening transduced cells to identify those which effects alteration of the phenotypic trait. The method of the present invention thereby render possible the identification of biologically active expression products derived from the random DNA library. These biologically active expression products can have

exerted their effect on the phenctypic trait in any of all possible steps in the cell's biochemistry which have influence on the phenctypic trait.

Withdrawal of the rejection is respectfully requested.

With respect to new claim 32, it is submitted that this claim is directed to screening steps which do not involve capture of the expression product with a known ligand. Since Kay *et al.* does not suggest any other methods of identifying TSAR's other than binding assays with a ligand of choice, the present invention is clearly not anticipated by Kay *et al.*

In new claim 33, it is specified that the phenotypic alteration screened for must be ascribable to the expressed ribonucleic acid(s) or peptide(s) affecting biologically functions of the cell which have influence on the preselected phenotypic trait. In other words, this claim specifies that the alteration screened for is not a mere binding of the expression product to a ligand of choice, but rather changes in a phenotypic trait (e.g., activity of an enzyme) which could be the result of the expression product interfering somewhere in the biochemical pathways relevant

to the phenotypic trait. Clearly, the disclosure of Kay et al. are not relevant as prior art under 35 U.S.C. § 102(e) for claim 33, since the "phenotypic trait" (i.e., expression of a TSAR) cannot in any way be the result of such an indirect mechanism.

In new claim 36 it is specified that the random expression products are confined to the intracellular compartment. Hence, binding assays as described in Kay et al. do not anticipate the invention as claimed in claim 36 for the simple reason that what is captured there is the direct expression product, whereas the present invention in the first round identifies cells wherein the expression product has effected a change and thereafter the expression product is retrieved from these cells. In other words, the method of Kay et al. requires that in order to identify cells expressing TSARs, it is essential that the cells carry these on their surfaces.

Claim 42 includes the limitation that the phenotypic trait screened for is one which is present in the identical cells even before they are transduced. This means that screening for heterologous material is not part of the claims invention.

However, Kay et al. specifically discloses screening for material of a heterologous nature, i.e., the "phenotypic trait" in Kay et al. cannot be found in non-transformed cells.

When reading the Examiner's rejections it has come to our attention that there were no rejections under 35 U.S.C. § 102 and 35 U.S.C. § 103 of claims 25-26.

Accordingly, new claims 34-35 are commensurate in scope with original claims 25-26, respectively.

Since claims 25-26 were not rejected in the first Office Action, we trust that this is still the case with new claims .

It is Applicants position that the Examiner has, however, failed to make out a *prima facie* case of obviousness by failing to provide the necessary motivation, other than through hindsight reconstruction of the invention, to combine the cited references. The only reason given by the Examiner to combine the cited references is because all of the references teach a key element of the claimed invention and that therefore it would be obvious to combining these references to produce the instant claimed invention (see page 14, last three lines.)

Even if there was motivation to combine the cited references (although it is Applicants position that there would not be such motivation for reasons discussed on more detail below) the cited art still fails to teach or suggest each and every feature claimed.

As already argued above, Kay *et al.* does not teach transduction of eukaryotic cells or that the method of Kay can result in transfection of one single vector per eukaryotic cell or a limited number of vectors per cell, in accordance with the presently claimed invention.

The Examiner has rejected claims 6-7, 12-14, 17-19 and 21 as being unpatentable over Kay et al. in view of Burke et al. and Wong et al.

As discussed above, Kay et al. discloses a method for expression of synthetic random DNA sequences in transfected cells, followed by subsequent screening of the cells for expressed peptides by use of a ligand of choice. What can be identified by means of the method of Kay et al. is therefore peptide binding partners to known ligands. Accordingly, this is also the aim of the methods disclosed in Kay 35 al.: To identify TSARs (Totally

Synthetic Affinity Reagents) which bind to ligands of choice against which the putative TSARs are screened.

The Examiner has cited Burke et al. for disclosing "...that the viral vector in an expression library contained <u>random</u> DNA sequences which encoded...". (Emphasis added). It is respectfully submitted that this is a misinterpretation of Burke et al., there is no mentioning therein of random DNA sequences. In contrast, Burke et al. discloses the preparation in yeast of polypeptides which are cross-reactive with HSV glycoprotein D (gD). It is specifically taught that the synthetic DNA constructs used in the preparation methods are derived from the naturally occurring gD encoding sequence, see the text in column 4, first full paragraph. Although modification to the gD encoding sequence are suggested, these are by no means random, in fact the word "random" or equivalents thereof does not seem to appear at all in the disclosure by Burke et al. Rather, the suggested modifications to the gD gene sequence are those which are known to enhance expression in yeast, those which are known to create or remove restriction sites, or those which substitute one or more amino

acids in the resulting polypeptide. It is indicated that the latter type of modifications may be made to enhance the immunogenicity, to facilitate conjugation, etc. Again, this is not random modifications but modifications of the gD gene sequence which serve a specific predefined purpose.

Hence, what can be extracted by the skilled artisan from Burke et al. is the use of DNA encoding leader, anchor, and signal sequences as well as glycosylated sequences. Random sequences are, however, not disclosed.

The reference by Wong et al. teaches an approach which could, judged superficially, bear some resemblance to the presently claimed invention. Wong et al. teaches retroviral transduction of cDNA libraries into cells derived from mammals followed by subsequent screening of these cells to identify those which have changed a phenotypic trait. The method of Wong et al. and other related methods (such as those disclosed in WO 95/04824 which was cited in the International Search Report) suffer a number of drawbacks. First of all, only naturally occurring expression products which produce an effect will be isolated in these methods.

Second a number of putatively interesting subsequences of naturally occurring expression products will never produce an effect, since these are part of larger expression products which may, due to sterical hindrance, be incapable of binding to possible targets of the subsequence.

The present invention provides for a method for identification of peptides and ribonucleic acids encoded by random synthetic DNA sequences which have been transduced into eukaryotic cells. The method does not rely on the provision of known ligands - on the contrary, the present invention allows for the identification of hitherto unknown ligands to the expressed random peptides or ribonucleic acids. The most powerful characteristic of the present invention is that it becomes possible to identify two important classes of substances: 1) Short peptides or ribonucleic acids which can serve as lead compounds in drug development, and 2) the molecule(s) with which the short peptides or ribonucleic acids interact inside the transduced cells.

Hence, the gist and crux of the present invention is, as mentioned above, the inventors' realization that short peptides and

ribonucleic acids are capable of interfering with biochemical processes which take place inside cells. The inventors therefore realized that if it is possible to obtain expression inside cells in a controlled manner of short random DNA sequences, the expression products thereof would be able to interfere with the biochemistry of the cells.

It would seem therefore, that a combination of Kay et al. and Burke et al. will not lead to the invention as claimed in the independent claims, since Burke et al. does not teach transduction. The transformation method used according to Burke et al. is introduction of plasmids "following the procedure of Hinnen et al.", see column 14, lines 65-68, i.e., transfection as in Kay et al. Furthermore, the disclosure in Burke et al. is silent when it comes to screening procedures essential in claims 32-33. Although Burke et al. in column 5, first full paragraph, discusses intracellular confinement of the expression product, this specific procedure is not applicable when trying to identify cells according to the method of Kay et al. (which requires a "connection" between expression product and the cell producing it). When Burke et al.

mentions intracellular expression, it is with a view to production of the gD analogues, not with a view to identification.

Although it would appear that the combination of Kay et al. and Wong et al. could theoretically arrive at the present invention (that is, by substituting the cDNA fragments of Wong et al. with the synthetic DNA fragments of Kay et al.), the skilled person would not contemplate this substitution of elements without having a motivation to do so. Without such motivation, the present invention is not obvious over the prior art. Since the aim of Wong et al. is to identify genes (that is, fully functional fragments of DNA which express naturally occurring polypeptides, see the title of abstract of Wong et al.), it is apparent that Wong et al. neither teaches nor suggests the feasibility in using synthetic DNA libraries in the search for biologically active peptides and ribonucleotides. By using random DNA libraries, the skilled artisan would know that the chances of identifying a gene would be reduced significantly when compared to using a cDNA library as a starting point.

In order for the skilled artisan to conceive the method of the present invention, it is thus necessary for him to realize that not only expression products of genetically derived material (such as the cDNA employed in Wong et al.) are capable of introducing indirect functional changes in cells but that also synthetically derived fragments are capable of introducing changes inside cells. Furthermore, the skilled person would also have to realize that additional advantages are achieved by using the present invention's "synthetic fragment approach":

a) the degrees of freedom in designing the transduction constructs are increments; it becomes possible to introduce anchor residues and other functional features in an otherwise random sequence and this provides for targeting of the expression products in a much more convenient manner than is the case for expression products of cDNA libraries where nucleotides encoding such functional features can only be introduced upstream or downstream to the cDNA.

b) The diversity of the expression products is increased because a properly constructed synthetic library includes many more unique sequences than does cDNA. This means that biologically active peptides and ribonucleic acids which are not naturally occurring (e.g., not part of cDNA encoded material) can be identified. Further, cDNA libraries are often deficient in long full-length cDNA fragments because reverse transcription is blocked by tertiary structures in the mRNA somewhere between the cDNA priming site and the upstream start codon, meaning that many fragments in a double stranded cDNA library lack the 5'-ends of the coding strand. This is not a problem according to the present invention, where all possible sequences can be introduced and expressed, including those which by chance are identical to such cDNA 5'-ends.

c) Because the number of nucleotides in the members of a synthetic DNA library can be controlled, it is possible to avoid masking of putative interactions between

expression products and cellular ligands. Expression products of a cDNA library could include putative binding sequences which are never detected because other parts of the expression product sterically masks the binding sequence.

d) When using embodiments of the present invention where random expression products are part of larger scaffold proteins, it is achieved that the random sequence is presented to the intracellular environment in a sterically optimal manner. This is not feasible when using cDNA libraries because of the large sizes and varying lengths of the cDNA expression products which will themselves possess tertiary structure.

e) It becomes possible to detect simple intracellular binding between the expression products and target molecules (this would presumably only be a rare incident when screening cDNA libraries, because the observed effects would largely be due to the native function of the cDNA expression product, see Wong et al. where the

interleukin 3 gene is identified due to its functionality).

f) When using relatively short synthetic random DNA fragments, it is easy to identify the particular part of the the expression product which is responsible for the observed alteration of the phenotype via a binding interaction. Often this particular part is simply identical to the full sequence of a short expression product. Again, when using cDNA libraries and assuming that a phenotype change is observed as a consequence of an "interaction effect," there would still remain a tedious job of identifying the specific part of the cDNA's expression product which is responsible for the effect.

g) Expression products which are found to produce positive results in a screening are in themselves useful as catching probes when screening for their ligands (for cDNA encoded expression products, this utility is of limited value since long expression products

statistically will bind to numerous irrelevant cross-reactive substances). At any rate, the ligands identified according to the present invention would not all be identifiable when using cDNA libraries, simply because the cDNA is less diverse than a synthetic library.

Without any suggestions in these directions in the prior art, the combination of the teachings of on the one hand Kay et al. and on the other Wong et al. to arrive at the presently claimed invention would not be contemplated by the skilled artisan.

It should also be noted that many researchers have expressed the concern that short expression products as those suggested in the present invention, would have too short a biological half-life inside the cells to have time to exert any detectable biological effects.

In conclusion, the Examiner has not demonstrated a *prima facie* case of obviousness of the presently claimed invention, since he has not indicated where the skilled person would find the motivation to transduce eukaryotic cells with vectors comprising

random DNA sequences and subsequently screen these for changes in a preselected phenotypic trait, let alone where the skilled person should find the motivation to screen for cells expressing such DNA fragments where the phenotypic change is ascribable to an indirect effect.

It should also be noted that more than approximately 9 months after the priority date of the present invention, others highly skilled in the art of molecular biology have filed patent applications which claims technology very much in line with the present application's disclosure, since WO 97/27212 and WO 97/27213 (assigned to Rigel, Inc. and Stanford University, respectively) disclose and claim subjected matter which is on many points identical to the subject matter of the present application. Furthermore, WO 98/32880 (Immusol) also relates to a technology which is based on the idea of intracellular expression of random DNA sequences and the same holds true for WO 98/39483 (Ventana). As evidence, we enclose copies of the front pages of the 2 latter applications (front pages from the first two were filed earlier

this year in the letter to the U.S. Patent and Trademark Office dated March 24, 1998).

The fact that these 4 later applications have been filed and prosecuted on a world-wide scale is, in Applicants' opinion, a clear indication that the present technology has been considered inventive by highly skilled persons also after the priority date of the present invention and this is a strong indication of the non obviousness nature of the invention. In view of the above submission, Applicants respectfully request favorable reconsideration.

The Examiner has rejected claim 7 as being unpatentable over Kay et al., Burke et al., and Wong et al., in view of Lund et al. Applicants submit that claim 7 is neither taught nor suggested by the cited references for reasons given above. Reconsideration and withdrawal of the rejection is respectfully requested.

In view of the above amendments and submissions it is Applicants' submission that the above-captioned application is now in condition for allowance. In the event there are any issues that can be expedited by telephone conference the Examiner is invited to call the undersigned at the number indicated below.

Respectfully submitted,

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